narrow the number of genes that orchestrate cartilage regeneration (and ear-wound healing) and may inform the likelihood of cartilage healing in patients.

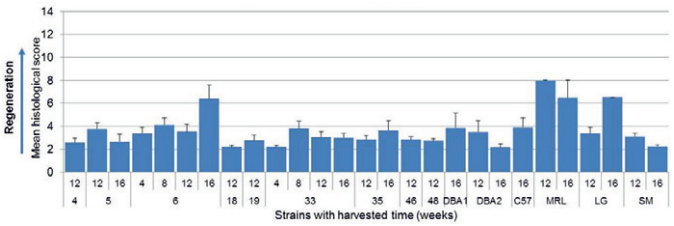

Fig. 2. Mean histological score for cartilage defects in healer and non-healer strains.

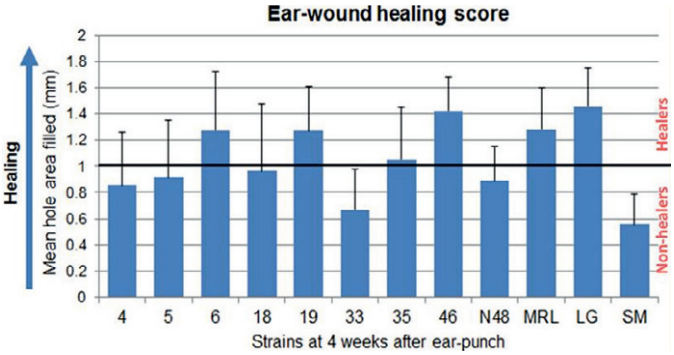

Fig. 3. Mean ear-wound diameter filled in healer and non-healer strains.

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IMPROVING hBM-MSCs DIFFERENTIATION IN A STRATIFIED SCAFFOLD FOR OSTEOARTICULAR REPAIRING

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Purpose: Osteochondral defect is followed by a fibrocartilage tissue repairing that present weak mechanical properties faced to native cartilage tissue. Until now, few strategies attempt to repair osteochondral defects; and generally, cartilage and bone tissues are study separately for repairing only focal lesions. However, some ways lead to obtain a stratified scaffold to repair the whole cartilage thickness or osteochondral defect by taking in account the depth-dependant variation of cells phenotype, and matrix composition and organization. Our work aimed at contributing to stratified tissue engineering with providing an original scaffolds composition of an hydrogel seeded with human Mesenchymal Stem Cells from Bone Marrow (hBM-MSCs). The biomaterial used is based on sodium Alginate supplemented with Hyaluronic Acid (Alg/HA) in a layer and with Hydroxyapatatite (Alg/Hap) in the other layer to mimic osteochondral matrix composition to target cells differentiation. Indeed, it is admitted that scaffold composition and mechanical properties influence cell phenotype.
Methods: A stratified hydrogel was build up by a simple and progressive spray of cellular gel suspension alternate with polyelectrolyte multilayer (PEMs) to weld layers. hBM-MSCs were embedded in an Alg/Hap gel, then sprayed and the biomaterial was gelled in a CaCl2 bath. Then, PEMs were built up on the hydrogel and a second hydrogel layer was constructed as previously, with an Alg/HA gel seeded with hBM-MSCs. We had evaluated cells comportment in the layered scaffolds cultured with a basic chondrogenic medium, without growth factor. In fact, we had checked cells proliferation by following cell cycle and explored matrix synthesis at 28 days of culture to evaluate hBM-MSCs differentiation, by histological and confocal microscopy observations.
Results: Previously, we built up stratified scaffold with Alg/HA in all layers and we had a good cohesion of layers up to 56 days of in vitro culture, and a matrix synthesis of which the type II collagen specific from cartilage tissue. We changed the bottom layer composition (first sprayed) to obtain different cells differentiation. We still observed good cohesion during the culture time. Moreover, we checked cells proliferation during 28 days, and we observed that cells stop it after their embedding in the hydrogel; indeed, they were most in G0/G1 phase. In addition, we checked extracellular matrix synthesis by histological, and by fluorescent labeling observed in confocal microscopy. In the two hydrogel compositions, we observed type II and type X collagens which are typically synthesized by chondrocytes and hypertrophic chondrocytes, respectively.
Conclusions: Here, we showed that we are able to construct bistratified scaffolds with two different hydrogel compositions and to induce a chondrogenic differentiation without biochemical or mechanical stimulations. In a near vision, challenge is to create a tristratified scaffolds to mimic more precisely cartilage layers and the sub-chondral bone layer by improving hydrogel composition to targeted cells differentiation. Moreover, PEMs are known to adsorb proteins as growth factor, this way can be explored to evaluate growth factor local delivery on cells phenotype. In a long term sight, those scaffolds can be implanted in osteochondral defect in animals to restore the tissue integrity to avoid its degeneration.
Acknowledgements: This work was supported by the project ANR06-BLAN-0197–01/CartilSpray, from the “Agence Nationale de la Recherche”, the “Conseil Regional de Lorraine” and the PIR-CNRS "vieillissement, longévité". Confocal microscopy pictures were obtained thanks to the “plate-forme imagerie cellulaire PTIBC – IBISA". We thank Astrid PINZANO for her help on histological study.

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THE PROTECTIVE EFFECTS OF GELATIN HYDROGEL–BONE MORPHOGENETIC PROTEIN-7 SYSTEM ON EXPERIMENTAL OSTEOARTHRITIS IN THE RABBIT KNEE

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Purpose: Bone morphogenetic protein 7 (BMP-7) has a strong anabolic effects on cartilage by stimulating the matrix synthesis. Therefore, BMP-7 is expected to be a target of pharmacotherapy for cartilage diseases. However, maintenance of effective concentration of proteins is difficult because usual proteins are metabolized immediately when direct administration into joints is performed. We previously reported the sustained releasing system of basic fibroblast growth factor had the therapeutic effects on experimental osteoarthritis (OA) in the rabbit knee. In this study, BMP-7 was administered into joints of OA rabbit models by sustained releasing system, and the protective effect on OA development was investigated.
Methods: Biodegradable gelatin hydrogel microspheres (GHM) were prepared for the controlled release of BMP-7. The average diameter was 70 μm.
^{125}I labeled BMP-7 contained in GHM was administered into the knee joints of normal rabbits to confirm the sustained release kinetics of the impregnated BMP-7 in the knee joint. The persistent radio-activities in the knee joint ($n=3$) were measured 1, 3, 7 and 14 days after administration.
Thirty Japanese white rabbits underwent unilateral anterior cruciate ligament transaction (ACLT).One μg BMP-7 with GHM (BMP-M group), one μg BMP-7 solution (BMP-S group) and PBS (PBS group) were injected into the ACLT knee joints once three weeks (a total of twice) from 4 weeks after ACLT. Ten weeks after ACLT, gross morphological (score 0–5) examinations were performed.
All ACLT knees were evaluated for gross morphological changes 10 weeks after ACLT and classified into 6 grades [Grade 1 (intact articular surface), 2, 3, 4a, 4b and 4c (severely degenerated articular surface)] by using India ink and the grade was scored from 0 to 5. Synovium and cartilage were harvested from the evaluated knee and examined histologically.
Results: The amount of labeled BMP-7 that remained in the knee joint was significantly larger in rabbits administered labeled BMP-7with GHM than in those administered labeled BMP-7-solution for 7 days. This showed that sustained-releasing of BMP-7 in the knee joint continued for over 7 days.
Severely damaged cartilage (Grade 4a, 4b, and 4c) was observed in 50% of PBS group, in 30% of BMP-S group and in 10% of BMP-M group. Averaged scores in PBS group, BMP-S group, and BMP-M group were 2.4, 1.5 and

0.9, respectively. The scores of cartilage damage in the BMP-M group were less severe than PBS group. The histological severity of OA in BMP-M group was significantly lower than PBS and BMP-S group according to the modified Mankin's histological grading. Remarkable inflammation or hyperplasia of synovium was not observed in any group.

Conclusions: Local administration of therapeutic agents to joints is reasonable in OA which is not systemic disease. The osmotic pumps were previously used for the maintenance of effective concentration of therapeutic agents in the joint of animal experiments. Biodegradable GHM was reported to achieve controlled release of growth factors in the condition that maintained the biological activity of growth factors. The present study showed this sustained releasing system of BMP-7 in the joint had protective effects on OA development in an animal model. This method might be a useful conservative treatment for human OA in the future.

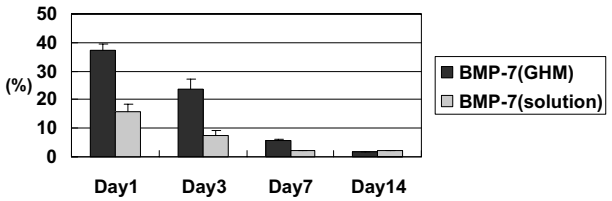

Fig. 1. In vivo release kinematics of BMP-7.

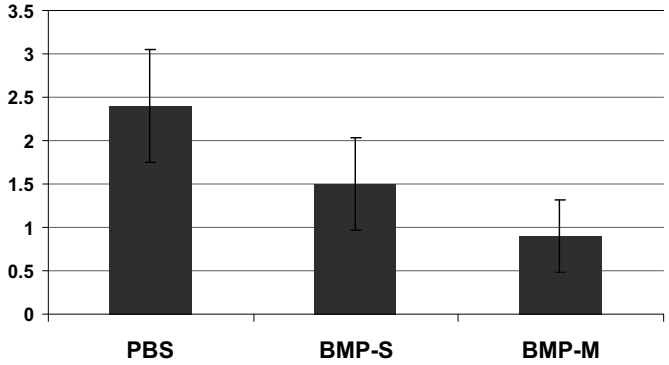

Fig. 2. Gross morphological evaluation (score).

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A SHORT TIME WINDOW TO PROFIT FROM IL-4 PLUS IL-10 ADDITION TO PROTECT CARTILAGE FROM BLOOD-INDUCED DAMAGE *IN VITRO*

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Purpose: Exposure of joint cartilage to blood can occur after joint trauma, during or after major joint surgery, or due to hemophilia. This ultimately leads to joint damage, having both the inflammatory characteristics of rheumatoid arthritis and the degenerative characteristics of osteoarthritis. It has been reported that interleukin (IL)-10 limits blood-induced cartilage damage *in vitro* and that IL-4 has some cartilage protective properties. Our aim was to study whether the combination of IL-4 and IL-10 has an additive effect on prevention of blood-induced cartilage damage when administered during (prevention) and after the onset (treatment) of blood-exposure.

Methods: Human full thickness articular cartilage explants were cultured for 4 days in the presence or absence of 50% v/v homologous blood, mimicking a joint bleed. Either IL-4 or IL-10 alone was added (0, 10, 30, or 100 ng/ml) during blood exposure (n = 9 donors). Additionally, a combination of IL-4 and IL-10 was added during blood exposure (prevention), as well as 2, 4, 8, 24, or 48 hours after start of blood-exposure (treatment) (n = 5 donors). After 4 days of culture the medium was refreshed and cartilage was cultured for an additional 12 days in the absence of additives to determine long-term effects of short-term blood-exposure and treatment. This mimics the *in vivo* situation where blood is cleared from a joint within approximately 4 days and where IL-4 and IL-10 have short half live times. Cartilage matrix turnover, in terms of proteoglycan synthesis, -release, and -content, was determined at day 16.

Results: Cartilage cultured in the presence of blood decreased proteoglycan synthesis rate, increased proteoglycan release, and decreased proteoglycan content as measured after 16 days of culture (all $p < 0.05$). This blood-induced damage to the cartilage matrix was limited both by IL-10 and IL-4 alone in a dose-dependent way. At 10 ng/ml, IL-10 and IL-4 improved proteoglycan synthesis rate with 41% and 125%, respectively; proteoglycan release decreased with 42% and 59%, and proteoglycan content increased both with 19% (all $p < 0.05$ compared to blood-exposure).

Most importantly, the combination of IL-4 and IL-10 (10 ng/ml) was more protective against damage caused by blood than IL-10 alone. The decrease of proteoglycan synthesis rate due to exposure to blood was further improved with 157%, and proteoglycan release decreased with 59% (both $p < 0.05$ compared to IL-10 alone).

When IL-4 plus IL-10 were added after the onset of blood-exposure, it appeared that it had to be administered within 8 hours after start of the bleeding to still be able to protect against blood-induced cartilage damage. At later time points addition was ineffective.

Conclusions: Besides IL-10, as shown previously, also IL-4 protects against blood-induced cartilage damage. This study demonstrates that the combination of IL-4 and IL-10 is clearly more effective than the individual components. Importantly, the protective effect is only evident when IL-4 and Il-10 are added within 8 hours after start of blood-exposure. This implies that treatment should be started within this time span to prevent blood-induced cartilage damage as a result of trauma or surgery.

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ENCAPSULATION IN ALGINATE BEADS RETAINS LONG-TERM IMMUNOMODULATORY PROPERTIES OF BONE MARROW MESENCHYMAL STEM CELLS

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Purpose: Mesenchymal stem cells (MSCs) are a source of pluripotent cells. They are able to self renew, differentiate into cells of mesoderm lineage and modulate the immune response. Here we focus on the immunomodulatory properties of MSCs for therapeutic use in osteoarthritis. We studied the possibility to encapsulate MSCs in alginate beads in order to achieve long-term immunomodulatory activity of MSCs and to place them in the jointcavity. We evaluated the expression of immunomodulatory factors by MSC upon stimulation with inflammatory factors when encapsulated in alginate compared to monolayer. Furthermore we studied the survival and differentiation capacity of MSCs after long-term encapsulation in alginate.

Methods: Human bone marrow MSCs of two different donors were encapsulated in 1.2% alginate or seeded in monolayer and after preconditioning for 48 hours they were stimulated by cytokines (50 ng/mL IFNγ, 50 ng/mL TNFα) for 24 hours. Each experiment was performed in triplicate. We studied the gene expression of MSCs compared to unstimulated control cultures. To evaluate the behavior after long-term encapsulation the above experiment was repeated after 30 days of preculture in alginate. We also measured the amount of DNA in a time course experiment and evaluated MSCs multi-lineage differentiation capacities after 1 and 30 days of encapsulation in alginate beads. Mixed model ANOVA was used to calculate statistical significance. A p-value <0.05 was considered statistically significance.

Results: Stimulation with IFNg and TNFa increased the expression of TNFα, IL1β, IL-6 (Fig1A), IDO (Fig1B) and COX2 in MSCs, whereas VEGF was downregulated ($p < 0.05$). TGFβ1 and TIMP2 didn't reach significance but showed a trend toward downregulation after cytokine treatment. The response of MSCs in alginate was similar to MSCs in monolayer culture. After 30 days in alginate the amount of DNA/alginate bead was unchanged, suggesting MSCs survive but do not proliferate in alginate. The cells still responded to inflammatory cytokines by increasing the expression of TNFα, IL1β, IL-6 (Fig1A), IDO (Fig 1B) and COX2 and